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Taft Stettinius & Hollister LLP
425 Walnut Street, Suite 1800 / Cincinnati, OH 45202-3957 / Tel 513 381 2838 / Fax 513 381 0205 / www taftlaw com
Cincinnati / Cleveland / Columbus / Dayton / Indianapolis / Northern Kentucky / Phoenix / Beijing

ROBERT A. BILOTT
513.357.9638
bilott@taftlaw.com

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TSCA Confidential Business Information Center (7407M)
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U.S. Environmental Protection Agency
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Re: Submission To TSCA 8(e) FYI Database Re: PFOA/PFOS

To TSCA 8(e)/FYI Database:

We are hereby providing the following information for inclusion in the TSCA 8(e)/ FYI databases with respect to PFOA/PFOS:

1. Seals, R., *et al.,* "Accumulation and Clearance of PFOA in Current and Former Residents of an Exposed Community," *Environ. Health Persp.* (doi: 10.1289/ehp.1002346) (online Sept. 22, 2010); and

2. C-8 Science Panel Status Report: "Patterns of Age of Puberty Among Children in the Mid-Ohio Valley in Relation to Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS)" (Sept. 30, 2010).

Very truly yours,

Robert A. Bilott

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Accumulation and Clearance of PFOA in Current and Former Residents of an Exposed Community

Ryan Seals, Scott M. Bartell, and Kyle Steenland

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Accumulation and Clearance of PFOA in Current and Former Residents of an Exposed Community

Author Names:

Ryan Seals[1], Scott M Bartell[2], Kyle Steenland[1]

[1]Department of Environmental and Occupational Health, Emory University, Atlanta, GA

[2]Program in Public Health and Department of Epidemiology, University of California, Irvine, CA

Corresponding Author:

Ryan Seals
1518 Clifton Road NE,
Atlanta, GA 30322

e. rseals@hsph.harvard.edu
p. 404-727-5368
c. 404-452-1960
f. 404-727-8744

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The authors declare they have no competing financial interests.

Abbreviations:

PFOA/C8 – Perfluorooctanoic acid

PPARα – Peroxisome proliferator-activated receptor alpha

Abstract

Background. PFOA is a perfluoroalkyl acid found in over 99% of Americans. Its health effects are unknown. Prior estimates of serum half-life range from 2.3 to 3.8 years.

Objectives. To assess the impact of years of residence and years since residence on serum PFOA concentration in a sample of current and former residents of six water districts in West Virginia and Ohio exposed to PFOA emissions from an industrial facility.

Methods. Serum samples and questionnaires, including residential history, were collected in 2005-2006. We modeled log serum PFOA (ng/mL) for current residents as a function of years of residence in a water district, adjusted for a variety of factors. We modeled the half-life in former residents via a two-segment log-linear spline in two water districts with high exposure.

Results. We modeled serum PFOA concentration in 17,516 current residents as a function of years of residence (R^2=0.68). Years of residence was significantly associated with PFOA concentration (1% increase in serum PFOA per year of residence), with significant heterogeneity by water district. Half-life was estimated in two water districts comprising 1,573 total individuals. Years-since-residing in a water district was significantly associated with serum PFOA, yielding half-lives of 2.9 and 8.5 years for water districts with higher and lower exposure levels, respectively.

Conclusion. Years of residence in an exposed water district was positively associated with observed serum PFOA in 2005-2006. Differences in serum clearance rate between low- and

high-exposure water districts suggest a possible concentration-dependent or time-dependent clearance process, or inadequate adjustment for background exposures.

Introduction

Perfluorooctanoic acid (PFOA, or C8) is a perfluoroalkyl acid used in the production of many fluoropolymers, including non-stick cookware, waterproofing, and flame retardants (Kennedy et al. 2004). Not naturally occurring, PFOA has been found in nature around the world, in multiple species, and in over 99% of serum samples obtained from the 2003-2004 National Health and Nutrition Examination Survey (Calafat et al. 2007; Houde et al. 2006). Despite recent regulatory and industrial efforts to phase out production and use by 2015, PFOA accumulates and persists in the environment, and human exposure is not expected to cease for some time.

Studies in rodents suggest that PFOA may be associated with many disease outcomes, including increased hyperplasias and benign tumors of the testicles, liver, and pancreas, low birth weight, decreased immune response, and decreased cholesterol (Hines et al. 2009; Kennedy et al. 2004; Lau et al. 2007). However, the appropriateness of the animal models has been called into question because of the wide range of clearance rates observed between and within species, and because of species-specific differences in the role of the PPARα-mediated effects of PFOA (DeWitt et al. 2009; Lau et al. 2007; Rosen et al. 2009). Human studies of PFOA have been thus far largely limited to cross-sectional studies and retrospective analyses of occupational cohorts. To date no clear health effects of PFOA have been established, but studies so far are sparse.

Average concentrations of 3.9 ng/mL (equivalent to parts per billion) were found in a nationally representative sample of US citizens in 2003-2004, with higher levels in males and whites (Calafat et al. 2007). Levels ranging from 100 to 5,000 ng/mL have been observed in occupational cohorts (Lau et al. 2007; Lundin et al. 2009; Olsen and Zobel 2007).

The current study population is derived from the C8 Health Project, which has been described previously (Frisbee et al. 2009). The C8 Health Project collected data on 69,000 current and former residents of the mid-Ohio valley who had been exposed to PFOA via contaminated drinking water. The average serum PFOA in this population was 82 ng/mL, with a median of 28 ng/mL (Steenland et al. 2009).

Establishing the rate of clearance of PFOA from the body is important for retrospectively determining lifetime exposure levels and for predicting future serum concentrations. PFOA is known to persist in human serum long after exposure has ceased, and is not metabolized in the body (Kennedy et al. 2004). Current estimates of serum half-life are derived from three primary sources. A study of 26 former employees of a manufacturing facility that produced PFOA, with a mean initial serum concentration of 799 ng/mL, estimated an average half life of 3.8 years (95% CI: 3.0-4.1), with individual half-lives ranging from 1.5 to 9.1 years based on a five year follow-up (Olsen et al. 2007). A more recent study of PFOA levels in 138 residents of a German community following implementation of charcoal filtration estimated a mean half-life of 3.26 years (range 1.03-14.67) (Brede et al. 2010). Finally, an ongoing study of 200 community residents living near a PFOA facility and part of the C8 Health Project (a subset of the same population studied here) were followed for one year and, based on multiple blood samples and a mean initial serum concentration of 180 ng/mL, exhibited a half-life of 2.3 years (95% CI: 2.1-2.4) (Bartell et al. 2009). Half-life estimates based on only one year of follow-up must be considered with caution. Preliminary results indicate that a traditional exponential decay model is sufficient for describing the clearance of PFOA from the body, despite earlier indications that clearance may occur in a time-dependent fashion in animals (Tan et al. 2007).

Our goal in this study was to estimate the effect of duration of residence on PFOA levels among current residents and to estimate the effect of years-since-leaving among former residents. The latter goal also involved estimating half-life. While use of cross-sectional vs. longitudinal data to estimate half-life is not optimal, it can provide useful information.

Methods

Data source. The C8 Health Project was conducted between August 2005 and August 2006, and collected health data from current and former residents of the study area using an extensive questionnaire and blood test, including the serum concentration of PFOA (n=69,030). A fuller description of the study has been published previously (Frisbee et al. 2009). The questionnaire, in addition to basic demographic information, included an extensive residential history beginning in 1980, and included information on water consumption source at each address (public/private, tap/bottled water). The questionnaire also queried individuals about behaviors including smoking, alcohol consumption, and vegetarianism.

Study participants. We identified individuals from the C8 Health Project who had consented to further follow-up and release of identifiable data to us, and who had provided residential history during the C8 Health Project (n=48,880).

As noted, our goal was to study the effect of duration of residence in a water district, and of years-since-leaving a water district, on PFOA levels measured in 2005-2006. Ideally for our purposes, water within a water district would have had a constant level of contamination over time, so that years of residence would reflect a constant exposure. In practice, PFOA emissions from the plant increased over time, peaking in the 1990s. In addition, different water districts are known to have different levels of contamination, largely due to distance from the plant (Steenland et al. 2009).

We first excluded individuals who had a self-reported history of employment by DuPont because of likely high exposure levels at the chemical plant (5%). We then excluded those who had a history of residence in more than one water district (25%); ever reported a private well as

their primary source of drinking water (11%); or reported intermittent residence in the water district of interest (9%). These exclusions were motivated by the desire for subjects to have continuous exposure to a single source of exposure, within a single contaminated water district. Because the limit of detection for serum PFOA was 0.5 ng/mL we also excluded individuals at or below this level (2%). Finally, we excluded individuals who reported overlapping residences in their residential history (3%). After all exclusions, 19,460 subjects remained for analysis.

Current residents. After the above exclusions. , we identified individuals who were residing in one of the six water districts on the date of interview and testing ("current residents"; n=17,516). The focus of the analysis of current residents was the effect of cumulative years lived in a water district.

Former residents. We studied a group of former residents to determine the effect of years-since-leaving on PFOA measured in 2005-2006 and to estimate half-life. We limited our analysis of former residents to the two water districts of Little Hocking and Lubeck, because these districts are hypothesized to have higher levels of exposure and half-life could be more reliably estimated. In addition to the above criteria for current residents, among former residents we excluded individuals with less than 2 years residence in a water district (11%), and a serum PFOA concentration lower than 15 ng/mL (28%). These criteria were used to limit the analysis to individuals who (1) had enough history in the water district to build up substantial levels of PFOA, and (2) had sufficiently high baseline PFOA concentrations, such that they had not reached background levels of PFOA by the interview date. The final cohort of former residents consisted of 643 Little Hocking residents and 1,029 Lubeck residents.

Statistical analysis.

General models without exposure terms of interest.

For all analyses, based on normalizing residuals for skewed data and in accordance with prior published results (Steenland et al. 2009), we modeled the natural logarithm of PFOA as measured in 2005-2006 as the outcome of a linear set of predictors. Variables considered as potential covariates were: sex, age, race (white vs. non-white), BMI, growing one's own vegetables, vegetarianism, alcohol consumption, current and former smoking, regular exercise, and use of bottled water as primary source of drinking water. These variables were all measured in 2005/2006, and had been used in prior analyses of PFOA levels (Steenland et al. 2009). Age, BMI, and date of interview were categorized as previously (Steenland et al. 2009).

For the analysis of current residents, with six water districts, using a backward selection process with a cutoff of 0.10 and without including duration of residence (our principal variable of interest) we created models individually for each of the six water districts, to determine which covariates would be included in final models. The backward selection process iteratively fit models, dropping the least significant covariate at each step until all were significant at the cutoff level of 0.10. In analyses with the six water districts combined, we added an indicator variable for water district to the model, which allowed us to determine the relative importance of residence in a particular water district, as well as the effect of having resided in that district.

This process was repeated for the analysis of former residents which was restricted to two water districts.

Analyses for duration of exposure

The goal of the first analysis was to estimate the relationship between duration of exposure to public water within a district and the measured serum PFOA level in 2005 or 2006.

For this analysis we considered only individuals residing in the six water districts on the date of interview and testing (current residents). Cumulative years in the water district was analyzed as both a continuous and categorical variable.

$$\ln(\text{PFOA}_{2005}) = \alpha + \beta \cdot \textit{CUM YEARS} + \boldsymbol{\delta} \cdot \mathbf{X} \quad [1]$$

where *CUM YEARS* represents the number of years lived in the water district, and $\boldsymbol{\delta}$ and $\boldsymbol{X}$ are parameter and covariate vectors.

Analyses by years since leaving (half-life analysis)

A second analysis was performed to estimate the half-life in former residents only (restricted to two water districts), via analyzing the relationship between the number of years since living in the water district and the measured serum PFOA level in 2005 or 2006, using the following model:

$$\ln(\text{PFOA}_{2005} - 5) = \alpha + \beta_1 \cdot \textit{YEARS SINCE} + \beta_2 \cdot \textit{CUM YEARS} + \boldsymbol{\delta} \cdot \mathbf{X} \quad [2]$$

where *YEARS SINCE* represents the number of years elapsed since residence in the water district, *CUM YEARS* is the number of years lived in the water district, and $\boldsymbol{\delta}$ and $\boldsymbol{X}$ are parameter and covariate vectors. This analysis was restricted to two water districts which had the highest levels, so as to avoid as much as possible the problem of background levels affecting our estimation of the elimination parameter (β). In this analysis, we subtracted background levels (5 ng/ml) from all subjects, and required that all subjects had at least 15 ng/ml PFOA in 2005.

Although performed on a single cross-sectional measurement of serum PFOA, rather than the more traditional longitudinal analysis of repeated measurements, the analysis by years since leaving can provide an approximation of the clearance rate of PFOA,. The number of years

elapsed since living in the water district was analyzed as both a continuous and categorical variable.

The half-life of a serum concentration describes the number of years required for the concentration required to reach one-half of the baseline level. Elimination of a substance from the circulatory system is usually described by a logarithmic process, where the concentration of the substance at time t (C_t) is related to the baseline concentration (C_0) by the time-dependent term $e^{-\lambda t}$, where λ is a positive decay constant, i.e. $C_t = C_0 e^{(-\lambda t)}$. This is called a first order elimination in which the rate of elimination is constant and does not depend on initial concentration. To obtain the half-life ($t_{1/2}$), we seek the time required such that C_t is ½ of C_0, or ($t_{1/2}$) such that $½ * C_0 = C_0 e^{(-\lambda t)}$. Rearranging, we have:

$$t_{1/2} = -\ln(1/2) / \lambda \quad [3]$$

The slope of the line describing the relationship between *YEARS* and ln(PFOA) is β, which is equal to λ in equation 3 above (also see below), and hence can be used to solve for the estimated number of years that would be required for the PFOA to fall by half. Since by model (2) we have predicted PFOA=$e^{\alpha} e^{\beta * \text{YEARS SINCE}} e^{\delta X}$, and, given that some change in *YEARS SINCE* will cut predicted PFOA in half, we have $½ = e^{\beta * (\text{YEARS SINCE1} - \text{YEARS SINCE2})}$, since the intercept and covariate terms cancel out. The change in YEARS SINCE is then the half-life, and taking the log of the last expression we regain model (3) and have

$$\ln(0.5) = \beta \cdot t_{1/2} \quad [4]$$

where β is equivalent to λ.

Graphing a scatter plot of log PFOA by YEARS SINCE, we found an apparent non-linear relationship using a LOESS non-parametric curve. We then modeled the relationship using a two-segment linear spline (Steenland and Deddens 2004). The spline is included in the model through the addition of a time-dependent variable that is zero prior to the knot and increases after the knot. Below is an example, with a knot at *YEARS SINCE=4*:

$$\ln(\mathrm{PFOA}_{2005} - 5) = \alpha + \beta_1 \cdot \mathit{YEARS\ SINCE} + \beta_2 \cdot \max[0,(\mathit{YEARS\ SINCE} - 4)] + \beta_3 \cdot \mathit{CUM\ YEARS} + \boldsymbol{\delta} \cdot \mathbf{X} \qquad [5]$$

The expression max[0,(*YEARS SINCE* – 4)] evaluates to 0 when *YEARS SINCE* ≤ 4 and equals (*YEARS SINCE* – 4) when *YEARS SINCE* > 4. The slope of the regression line is therefore β_1 prior to the knot, and (β_1+β_2) after the knot. We chose the knot based first on visual inspection of the relationship between years elapsed and ln(PFOA), to determine the likely region of interest, followed by an iterative procedure where we picked the knot with the best model likelihood.. We used an F-test to test the increase in the goodness-of-fit in the spline model over the linear model. Outliers with absolute studentized residuals greater than 3 were discarded (0.5%).

Prior work has suggested that half-life estimates after truncation to account for near-background levels can introduce bias (Michalek et al. 1998). We performed a sensitivity analysis using various truncation values (serum PFOA concentrations below the truncation value were discarded) to assess the robustness of our half-life estimates, retaining the same models and knot locations as in the initial analysis with truncation at 15 ng/mL. In all analyses, 5 ng/mL was subtracted after truncation and before regression.

The method for ascertaining the serum concentration of PFOA has been described previously (Frisbee et al. 2009). This study was approved by IRBs at all C8 Science Panel

institutions, and all applicable requirements for human research were met. All participants gave written informed consent to participate in the C8 Health project; consent procedures have been described previously (Frisbee et al. 2009). All analyses were performed using SAS v9.1 (Cary, NC). Images were generated using PASW v17.0 (Chicago, IL).

Results

Median levels of PFOA for current and former residents are shown in Table 1. In residents still residing in the six water districts at the time of the interview, differences in serum PFOA levels were apparent across water districts, sex, use of bottled water, growing own vegetables, smoking history, and date of testing, similar to results reported previously for the entire C8 Health Study cohort (Steenland et al. 2009). The subset of 1,672 former residents had higher PFOA levels than the current residents because this subset was limited to residents of Little Hocking and Lubeck, the two highest-exposed water districts.

Current Residents. Figure 1 displays the relationship between cumulative years of residence in the six water districts and the natural logarithm of serum PFOA (ng/mL), in individuals reporting residence in one of the six water districts on the date of interview in 2005-2006 (current residents). The positive slope is significant at the $p<0.001$ level for all six districts. The effect of cumulative years is reasonably linear with ln(PFOA).

The results of the full model after backward selection, with an indicator variable for water district, are shown in Table 2. The R-squared for the full model was 0.68. Water district residence explained the majority of the variance (partial R-squared), with residence in Little Hocking alone accounting for 39.4%. After residence, cumulative years of residence explained 1.5% of the variance. Previously observed associations were also replicated: higher levels in males, a U-shaped relationship with age, higher levels in current vs. never smokers, and higher levels in those who grow their own vegetables (Steenland et al. 2009).

The average increase in PFOA levels for each year of residence in a water district was 1.2% (95% CI: 1.1-1.4%). However, because exposure levels are known to be different between

water districts, and because median serum PFOA levels differed so greatly by water district, we fit models for each water district separately to yield district-specific effects of cumulative residence (Table 3; F-test for six interaction terms significant at $p<0.001$). As expected, districts with the highest exposure levels display the largest relationship between years of residential history and serum PFOA. In Pomeroy and Mason County, the districts with the lowest exposures as measured in current residents, the effect of years of residence was least.

Former residents. Using a two-segment linear spline regression with the same variables as above, we obtained estimates for the effect of years elapsed since residence on ln(PFOA) for the two segments of the spline curve. Based on visual inspection of a LOESS curve and goodness-of-fit statistics comparing various possible knots, we chose four years as the knot for Little Hocking and nine years as the knot for Lubeck. Figures 2a and 2b show the plots and fitted lines for the two water districts. An F-test for reduction in model error after moving from 1 segment (standard linear regression) to 2 segments was significant at $p<0.001$ for both water districts, and overall model fit (R-square) increased by 4% in Little Hocking and 3% in Lubeck after inclusion of the spline. A three-segment linear spline was not a significantly better fit to the data in either water district.

The estimated half-lives and percent change in PFOA by year for the two line segments in each water district are shown in Table 4. Because the half-lives are calculated from the slope, they represent the half-life that would result if the instantaneous rate of clearance were to continue indefinitely. The shallower line after the knot likely reflects either the gradual slowing of PFOA clearance over time (Little Hocking) and/or the decline of low exposures to near background (15 ppb) in the case of Lubeck. For example, an individual from Little Hocking with an initial serum PFOA of 55 ng/mL would have a concentration of 21 ng/mL after four years

(21% reduction per year), and a concentration of 15 ng/mL four years later (8% reduction per year). The resultant half-lives for Little Hocking were 2.9 and 10.1 years for the two spline segments, while for Lubeck they were 8.5 years in the initial spline segment and undefined for the second segment (the estimated parameter was 0.0021, or approximately 0, indicating no further decrease in PFOA levels over time). Our estimated half-lives were sensitive to the truncation cutpoint we used, below which subjects were excluded on the basis that they were near background levels. Table 5 displays various half-life estimates for the first spline segment in Little Hocking and Lubeck after various truncation values were applied. Little Hocking half-life estimates ranged from 2.5 to 3.0 years, while in Lubeck estimates ranged from 5.9 to 10.3 years. At all truncation values the half-life in Little Hocking was lower than in Lubeck, with larger discrepancies at higher truncation values. Because former Lubeck residents had lower serum PFOA concentrations, more individuals were discarded from Lubeck at all truncation values, with the discrepancy larger at higher values.

Our estimated half-lives were also sensitive to the amount we subtracted off of our PFOA levels, a subtraction designed to eliminate background levels in estimating half-life. To test the robustness of our estimate to this subtraction, we performed a similar analysis that eliminated all individuals below 15 ng/mL in 2005, and subtracted 15, rather than 5, from their measured value. Results were similar to our original results: the estimated half-life for the first four years of clearance in former residents of Little Hocking was 3.0 years, while for Lubeck (for the first nine years) it was 9.4 years.

PFOA clearance appears to be sex-dependent in rats (with a much longer half-life in males), but not in monkeys (Lau et al. 2007). In our data for Little Hocking, males were associated with a faster rate of clearance ($p=0.02$), but only in the first four years. Annual

reduction in serum PFOA was 27% in males vs. 18% in females. The effect was non-significant after four years. However, prior longitudinal analyses of 200 residents in Little Hocking and Lubeck found no sex differences in half-life (Bartell et al. 2009). We did not observe sex differences in former residents of Lubeck.

Discussion

We found a significant positive association between years of residence in an exposed water district and serum PFOA, with an average of 1% increase per year of residence. Lower levels of serum PFOA in former vs. current residents residence has been demonstrated in this cohort previously (Steenland et al. 2009), but this analysis now demonstrates a significant trend within current residents (those still residing in exposed water districts in 2005-2006 based on their prior residential history). We also found a more substantial relationship between PFOA and years of residence in water districts closer to the industrial facility, as expected. After water district, years of residence accounted for the greatest variance in the fitted model. These findings provide preliminary justification for possible use of residential history as a proxy for prior exposure in epidemiologic studies.

In former residents the main finding from our analysis was that the use of a two-segment spline increased the model fit and better approximated the observed relationship than a simple linear model. In both water districts, an apparent nonlinear relationship resulted in a significantly lower clearance rate after the knot of either 4 or 9 years. If our assumptions are correct this implies that a simple first order elimination model may not hold, and that the rate of elimination may be concentration-dependent or time-dependent. We feel that the results suggest both a concentration- and time-dependent relationship because the time factor is the same for both Little Hocking and Lubeck (years since former residence), but exposure was lower in Lubeck. However, the apparent time-dependent relationship could also be due to the concentration decrease over time. It is interesting that the rate of decay (slope) of the second linear segment for Little Hocking is similar to the rate of decay for the first segment for Lubeck, at similar concentration levels. In our cohort, former residents of Little Hocking had PFOA levels roughly

twice as high former residents of Lubeck. If serum clearance were concentration-independent the equation describing the relationship between PFOA and years living in the district and years elapsed since living in district would be the same in Little Hocking and Lubeck. Furthermore, within each water district the decay in ln(PFOA) would be linear rather than exhibiting a lower slope at lower concentrations.

As in prior studies of this population, we observed decreasing serum concentrations across dates of testing (Steenland et al. 2009). This may be due to behavior modification as the putative health effects of PFOA became publicized, both in increased bottled water usage and decreased tap water consumption. We observed a slight increase in reported bottled water usage over the testing period, and Little Hocking was offering free bottled water to individuals. Additionally, it is plausible that those who tested earliest were those who lived closer to the industrial facility and in more highly exposed water districts. However, adjusting for date of testing did not significantly alter any of our parameters of interest.

Prior studies in humans have found no difference in clearance rates between men and women, but animal studies have suggested that females may be more effective clearers of PFOA (Bartell et al. 2009; Brede et al. 2010; Lau et al. 2007). Harada et al. demonstrated in moderately exposed city-dwellers that renal excretion rates in both males and females were negligibly small, but that female clearance may be age-dependent (Harada et al. 2005). In our cohort we observed lower PFOA levels in females, an observation consistent with prior studies in this cohort and others (Calafat et al. 2007; Steenland et al. 2009). However, we observed a significantly faster clearance rate in men in the initial years of former Little Hocking residents. This calls into question the assumption that lower levels in females are due to faster rates of clearance, but we cannot rule out that the apparent sex effect is due to concentration.

This study has three major limitations. The first is the cross-sectional nature of the analysis. Particularly in the estimation of half-life, this limited our ability to draw inferences from the analysis. Although cross-sectional half-life estimation has been used in an analogous setting for urinary bisphenol A after fasting (Stahlhut et al. 2009), traditional half-life studies follow individuals over time, allowing researchers to compare serum concentration at any point in time to the initial concentration. Cross-sectional analyses must rely on model-based estimation of the initial concentrations instead of directly observed values. Our regression model included years of residence in the contaminated water district, sex, age, growing own vegetables, smoking, and consuming bottled water. We relied on recall via questionnaire to develop prior residential history. In addition to missing and incomplete data (gaps in residential history, which led to the exclusion of some subjects from the analysis), there is the possibility that individuals misreported their water district and/or years of residence.

The second major limitation is the implied assumption that exposure was uniform within a water district, both between individuals and over time, which we know to be false. Although we excluded individuals who were employed by DuPont or who reported private well use to limit the heterogeneity of the population, individual exposure was undoubtedly varied based on geographical location, individual behavior, and other uncontrollable factors. Also, we know that PFOA emissions from the plant were not constant over time and peaked in the late 1990s, but we were unable to account for this without quantitative estimates of annual water system concentrations. Further studies of this population will make use of advanced exposure models that account for both individual and temporal variations in exposure.

A third major limitation of our analysis is the potential bias introduced by the exclusion of participants with serum levels below 15 ng/mL. Truncation below a fixed concentration

threshold is known to introduce bias in half-life estimates for longitudinal data (Michalek et al. 1998), and is likely to have a similar effect in cross-sectional analyses. Although restricting the analysis to individuals with PFOA serum concentrations below 15 ng/mL avoids one type of bias (overestimation of half-lives among participants whose PFOA serum concentrations are no longer in decline by the time of the serum sample), it is likely to introduce another type of bias resulting in overestimation of half-lives, because excluded participants are likely to have shorter half-lives on average than retained participants. Our sensitivity analysis using different truncation values resulted in a smaller range of values for the more highly exposed residents of Little Hocking, while the half-life in former Lubeck residents was more sensitive to the truncation value. Notably, Lubeck residents tended to have lower concentrations, so truncation at all values resulted in more individuals discarded from the Lubeck analysis, with a progressively larger difference at higher truncation values.

A minor limitation of this study was the inability to differentiate between variable exposure levels and accumulation due to constant exposure. However, because emission levels and predicted water concentrations were known to be variable over the study period, peaking in the late 1990s, we feel that some of the annual increase as shown by the significance of years of residence is likely due to increasing exposures, rather than approach to a steady-state (Paustenbach et al. 2007). Further work will be done with exposure estimates that vary by year and location of residence.

These results suggest that the half-life for PFOA lies between the previously reported estimates of 2.3 and 3.8 years for more highly exposed individuals, but that serum clearance of PFOA may be concentration-dependent.

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Tables

Table 1. Median PFOA (ng/mL) in 2005-2006 (N) for current and former residents.

Variable	Current	Former[a]	Variable	Current	Former
Total	33.0 (18,068)	36.5 (1,672)	Drink Bottled Water		
			Yes	55.5 (532)	77.7 (70)
Water District			No	32.9 (17,031)	36.2 (1,518)
Belpre	31.0 (1,999)	--			
Little Hocking	241.0 (3,154)	60.6 (643)	Grow Own Vegetables		
Lubeck	69.4 (3,131)	31.0 (1,029)	Yes	38.3 (4,885)	39.4 (228)
Mason County	12.4 (5,052)	--	No	31.5 (13,183)	35.9 (1,444)
Pomeroy	11.8 (640)	--			
Tuppers Plains	36.4 (4,092)	--	Vegetarian		
			Yes	37.1 (168)	38.0 (13)
Sex			No	33.0 (17,900)	36.5 (1,659)
F	31.0 (9,330)	35.1 (872)			
M	34.9 (8,738)	37.3 (800)	Currently Consume Alcohol		
			Yes	37.1 (6,108)	35.8 (845)
Race			No	30.6 (11,216)	36.8 (784)
White	32.9 (17,579)	36.5 (1,637)			
non-White	35.2 (489)	35.1 (35)	Smoker		
			Current	28.1 (3,473)	35.5 (353)
BMI			Former	38.0 (3,968)	37.7 (364)
<24	32.3 (6,328)	37.1 (575)	Never	33.1 (10,627)	36.6 (949)
24-26	35 8 (3,454)	37.5 (354)			
27-29	34.8 (3,099)	36.9 (315)	Date of Testing[b]		
≥30	31.1 (5,187)	33.6 (428)	First two months	59.7 (2,068)	39.2 (134)
			Second two months	51.8 (2,377)	37.8 (218)
Regular Exercise			Third two months	34.0 (5,389)	34.8 (514)
Yes	36.8 (5,794)	36.6 (645)	Fourth two months	28.7 (4,537)	35.3 (481)
No	31.2 (12,274)	36.4 (1,027)	Fifth two months	21.5 (2,431)	40.1 (147)
			Last two months	17.0 (1,266)	38.0 (178)

[a]Former residents limited to individuals in Little Hocking and Lubeck with >2 years residence and >15 ng/mL PFOA.

[b]Dates of testing: 8/1/05-9/30/05, 10/1/05-11/30/05, 12/31/05-1/31/06, 2/1/06-3/31/06, 4/1/06-5/31/06, 6/1/06-8/31/06

Table 2. Multivariate linear regression results[a] (R^2=0.68), current residents (n=17,516[b]).

Variable	Predicted change in PFOA (% from referent)	Log change in PFOA	95% CI		Variance (%) in ln(PFOA) (partial R^2)
Cumulative years of residence	1%	0.012	0.011	0.014	1.5%
Sex, Female	-12%	-0.133	-0.153	-0.112	0.9%
Age					
< 20	Referent	--	--	--	--
20-29	-23%	-0.261	-0.302	-0.220	0.9%
30-39	-12%	-0.126	-0.169	-0.083	0.2%
40-49	-1%	-0.006	-0.045	0.033	0.0%
50-59	4%	0.042	0.004	0.080	0.0%
60-69	18%	0.167	0.126	0.208	0.4%
>70	27%	0.236	0.192	0.279	0.6%
Grow vegetables	11%	0.106	0.083	0.129	0.4%
Smoking					
Never	Referent	--	--	--	--
Current	12%	0.117	0.088	0.146	0.4%
Former	1%	0.012	-0.016	0.039	0.0%
Bottled water	-26%	-0.301	-0.361	-0.241	0.5%
Water district					
Tuppers Plains	Referent	--	--	--	--
Belpre	-12%	-0.129	-0.166	-0.091	0.3%
Little Hocking	495%	1.783	1.750	1.815	39.4%
Lubeck	82%	0.600	0.566	0.634	6.5%
Mason County	-64%	-1.018	-1.047	-0.989	21.1%
Pomeroy	-67%	-1.102	-1.161	-1.043	7.1%

[a]Model also adjusted for date of visit

[b]552 individuals missing covariate data (Bottled water=505, Smoking=66).

Table 3. Effect of years of residence on serum PFOA by water district, with district-specific model fit.

Water District	N	Model R^2	Variance (%) in ln(PFOA) (partial R^2) explained by years of residence	% Change in Predicted PFOA by Year of Residence	95% CI	
Tuppers Plains	3,986	0.18	3.2%	1.7%	1.4%	2.0%
Belpre	1,940	0.10	0.6%	0.7%	0.3%	1.1%
Little Hocking	3,054	0.10	0.8%	1.2%	0.7%	1.7%
Lubeck	3,044	0.23	3.6%	1.9%	1.6%	2.3%
Mason County	4,885	0.08	0.6%	0.6%	0.4%	0.9%
Pomeroy	607	0.12	0.5%	0.5%	-0.1%	1.1%

Table 4. Multivariate linear regression results, former residents of Little Hocking (n=602[a]) and Lubeck (n=971[b]).

Variable	Estimated Half-Life (years)	% Change in PFOA by Year	95% CI	
Little Hocking				
Years Elapsed, <4	2.9	-21.4%	-26.1%	-16.5%
Years Elapsed, >4	10.1	-7.6%	-18.1%	6.4%
Years of Residence	--	1.9%	0.8%	3.0%
Lubeck				
Years Elapsed, <9	8.5	-7.8%	-9.1%	-6.5%
Years Elapsed, >9	n.a.[c]	0.2%	-3.3%	3.8%
Years of Residence	--	2.5%	1.8%	3.1%

[a]Models also adjusted for sex, age, growing own vegetables, smoking, and consuming bottled water.
[b]Final analysis numbers due to missingness in smoking history and consumption of bottled water.
cParameter (0.002) yields a positive half-life not significantly greater than zero.

Table 5. Sensitivity analysis for half-life after various truncation cut points of serum PFOA.

Value (ng/mL)	Half-life, years (95% CI)[a]	
	Little Hocking	Lubeck
20	3.0 (2.4–4.0)	10.3 (8.7–13.1)
15	2.9 (2.3–3.8)	8.5 (7.1–10.1)
10	2.5 (2.0–3.3)	6.6 (5.8–7.8)
5	2.7 (2.1–3.9)	5.9 (5.1–7.1)

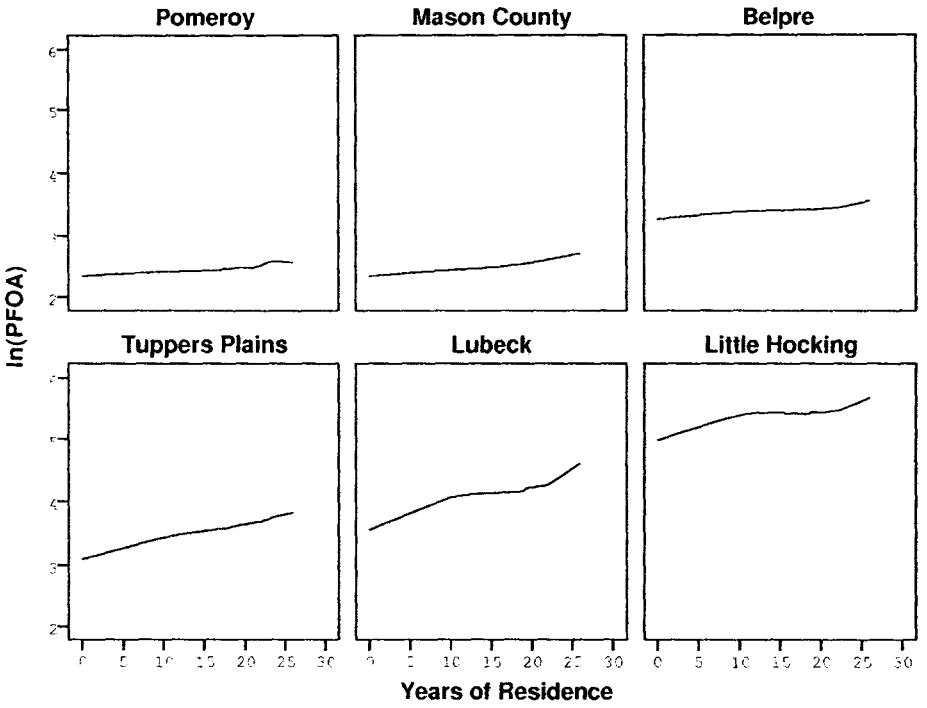
[a]Models also adjusted for sex, age, growing own vegetables, smoking, and consuming bottled water

Figure Legends

Figure 1. Plots of natural logarithm of PFOA (ng/mL) by cumulative years of residence in a water district, current residents, LOESS regression.

Figure 2a. Predicted decay of serum PFOA concentration based on half-lives estimated from former Little Hocking residents in discrete segments of less than 4 and greater than 4 years since living in Little Hocking (solid line; adjusted for covariates), and LOESS regression (dashed line; unadjusted for covariates).

Figure 2b. Predicted decay of serum PFOA concentration based on half-lives estimated from former Lubeck residents in discrete segments of less than 9 and greater than 9 years since living in Lubeck (solid line; adjusted for covariates), and LOESS regression (dashed line; unadjusted for covariates).



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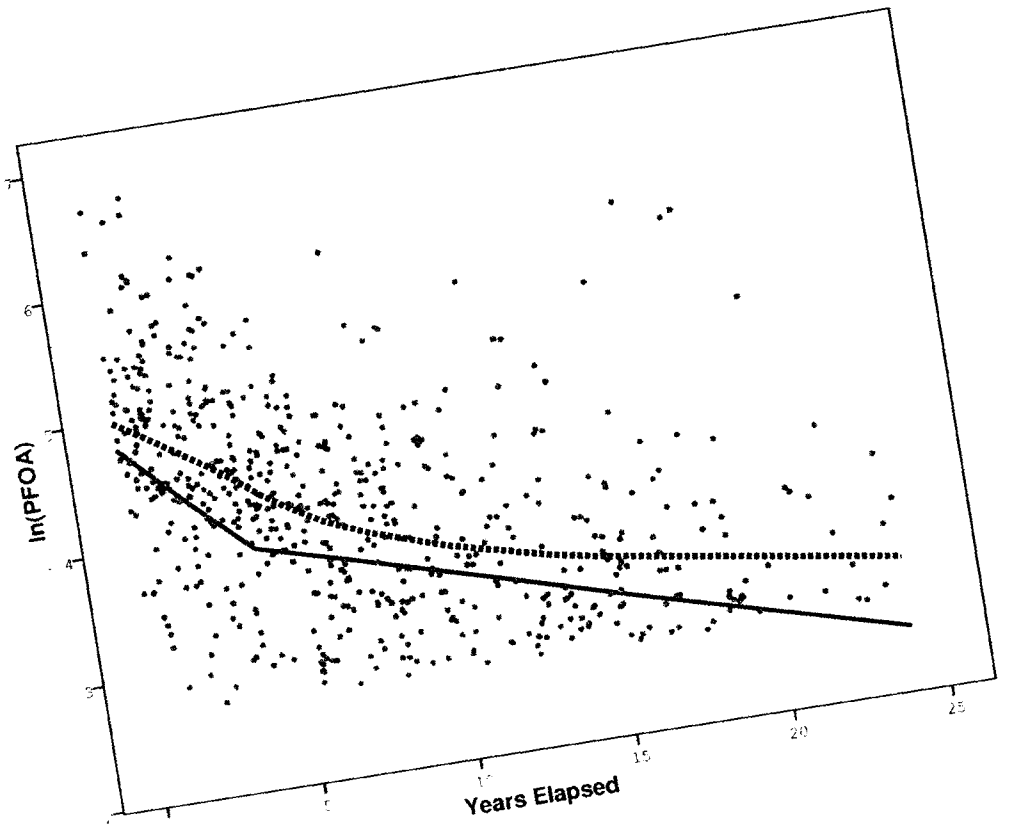

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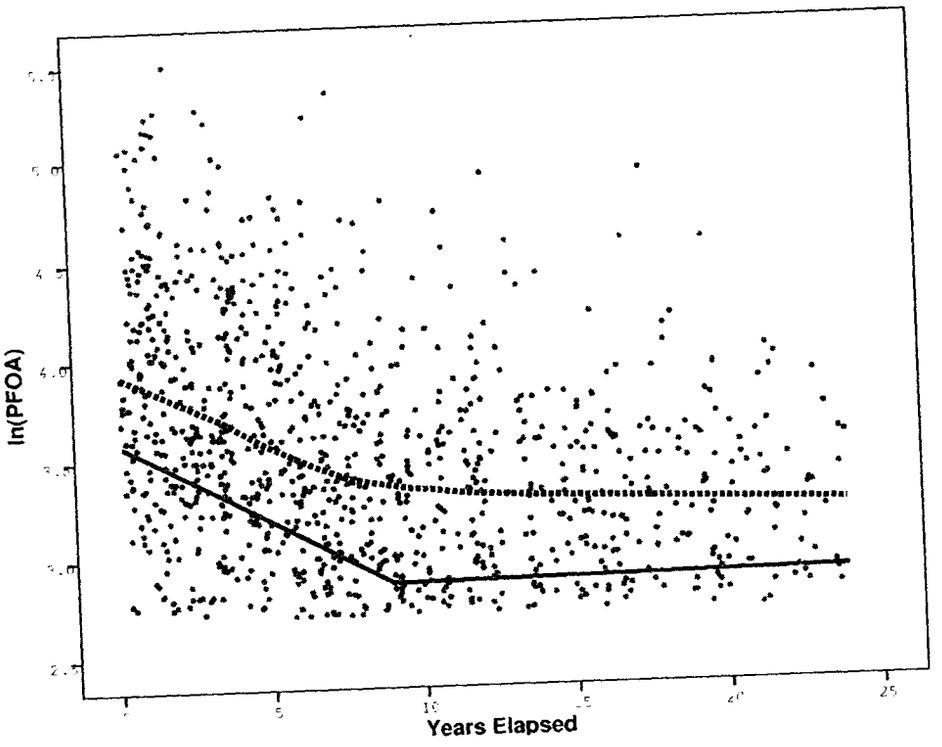

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Status report

Patterns of age of puberty among children in the Mid-Ohio Valley in relation to Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS)

The C8 Science Panel (Tony Fletcher, Kyle Steenland, David Savitz)

Sept 30 2010

This status report summarizes the findings of a statistical analysis of the relationship between levels of perfluorooctanoic acid (PFOA, also called C8), and perfluorooctane sulfonate (PFOS) measured in the blood serum of the children who participated in the C8 Health Project, and puberty. A full report of these findings will be submitted to a peer-reviewed scientific journal.

Introduction.

It has been suggested that some polyfluoroalkyl compounds (PFCs) may alter animal sexual maturation. The aim of this study was to examine the relationship between levels of two PFCs – PFOA (or C8) and PFOS – with puberty based on sex hormone levels and self-reported onset of menstruation. We used data from the C8 Health Project supplemented with detailed date of birth available from those who consented to be in the Science Panel studies.

Methods.

Among the young participants aged 8-18 at the time of the C8 Health Project survey (2005-2006), we examined data for 3076 boys and 2931 girls, all residents for at least a year in the six water districts which had been contaminated with PFOA. They were classified as having reached puberty at the time of interview based on either sex hormone blood levels (testosterone >50 ng/dL or free testosterone >5 pg/mL for boys, and estradiol >20 pg/mL for girls), or having reported that they had started menarche (periods). Statistical models estimated the chance of reaching puberty in relation to PFOA, and PFOS levels, while controlling for other potential explanatory factors. From these models, we could also estimate the average age of reaching puberty for children with different exposure levels, and present the difference (earlier or later) in days of reaching puberty between different exposure groups. We divided the population into four equal groups (quartiles) by exposure to PFOS and PFOA.

Results.

The mid-point of PFOA and PFOS serum levels were 26 and 20 ng/mL in boys, and 20 and 18 ng/mL in girls. For boys, there was a clear relationship of reduced odds of having reached puberty with increasing PFOS (delay of 190 days between the highest and lowest quartile), but not PFOA. For girls, higher exposure to either PFOA or PFOS was associated with reduced odds of having reached puberty. The highest PFOA group had an average age of puberty 130 days later than the lowest exposure group, and for PFOS, the delay was estimated as 138 days comparing the highest and lowest exposure group. These results are consistent in direction and magnitude with one published study which suggested delayed puberty (also measured as self-reported menarche) in relation to PFOS exposure in girls, and in contrast to another study which reported younger puberty (measured as breast maturation) in girls in relation to PFOA exposure.

Conclusions.

Delays of puberty have been observed in this population correlated with PFOS in boys and PFOA and PFOS exposure in girls. Caution is needed in interpreting these results, due to the fact that blood PFC levels and puberty status based on sex hormone levels were determined at the same time, and menarche was self-reported. For example, it may be that growth changes associated with puberty lead to changes in PFOA and PFOS blood levels, rather than these compounds having any effect on age at puberty. Further work is planned to investigate patterns of puberty by age in relation to exposure prior to puberty.

Taft/

Taft Stettinius & Hollister LLP
425 Walnut Street, Suite 1800
Cincinnati, Ohio 45202-3957